THE EFFECT OF LABORATORY COLONIZATION ON THE VECTOR-PATHOGEN INTERACTIONS OF EGYPTIAN *CULEX PIPIENS* AND RIFT VALLEY FEVER VIRUS*

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Abstract. Field and laboratory findings implicated *Culex pipiens* as a vector of Rift Valley fever (RVF) virus during the 1977–1978 epizootics/epidemics in Egypt. This study evaluated changes in infection and transmission rates, and viral titers in F_1 through F_{16} generation *Cx. pipiens* mosquitoes orally infected with RVF virus. Infection and transmission rates of RVF virus by this species changed significantly during the colonization process. However, the ultimate viral titers of either the transmitting or the infected nontransmitting mosquitoes were not affected by the colonization process. Following ingestion of virus, *Cx. pipiens* could be separated into three distinct subpopulations, an uninfected group and two types of infected mosquitoes—transmitters and nontransmitters. Transmitters contained significantly more virus (approximately 100-fold) than nontransmitters. These results demonstrated that not every infected female mosquito should be considered a competent vector, even if the species (population) is known to be a primary vector. Transmission was also accomplished by probing mosquitoes which were unsuccessful in obtaining a blood meal. These data document the long-held suspicion that vector competence studies based upon laboratory-colonized specimens may not represent the field situation.

Until recently, Rift Valley fever (RVF) was considered to be primarily a disease of domestic animals in sub-Saharan Africa, while in humans it was considered serious, but rarely fatal. The virus was first reported north of the Sahara Desert in 1977 when it caused an epizootic/epidemic in the Nile Delta of Egypt which resulted in considerable animal and human mortality.[1-3] Field and laboratory findings implicated *Culex pipiens* L. as a vector of RVF virus.[4]

One of the original objectives of this research

was to examine variation, if any, in the vector potential of different geographic populations of Egyptian *Cx. pipiens* for RVF virus. However, it was discovered that this objective could be achieved only by using field-collected and not laboratory-reared material. Because of the nonavailability of a continuous supply of field-collected mosquitoes, this study focused on the effects of laboratory colonization on the vector-pathogen interactions of subsequent filial generations of field-collected Egyptian *Cx. pipiens*. Specifically, we examined the infection and transmission rates, and various parameters of viral replication in these mosquitoes.

MATERIALS AND METHODS

Mosquitoes

The *Cx. pipiens* used in this study originated from approximately 500 adults and larvae collected on 10–11 March 1981 from several sites in the village of Abu Heif, Sharqiya governorate, Egypt. Adult mosquitoes were collected by aspiration during night human and animal bait collections, while larvae were collected from small stagnant pools of water. Both stages were trans-

ported to Frederick, Maryland, in chests cooled with wet ice. Voucher specimens were deposited in the U.S. National Museum of Natural History Smithsonian Institution.

Two separate insectaries were used for mosquito rearing. Experimental mosquitoes which had been exposed to RVF virus were maintained in a P3+ biosafety laboratory, while stock colonies were maintained in an insectary in another building.

To insure that members of the field-collected parental generation were not transovarially infected with RVF virus, we monitored all hamsters used as blood meal sources for four consecutive filial generations. All mosquitoes were reared on a pooled basis in bioclimatic chambers at 26°C with a relative humidity (RH) of 70–80% and a photoperiod of 15 hours of light and 9 hours of darkness. Adults were maintained in 30.5 × 30.5 × 30.5 cm screened cages that were draped with moist towels and covered with clear plastic to maintain humidity. Apple slices and a 5–10% sucrose solution were provi~..d as a carbohydrate source. Hamsters served as the source of blood for the females. Larval rearing was similar to the methods of Chapman and Barr.[5] Larvae were reared in 29 × 19 × 5 cm plastic pans containing 1 liter of continuously aerated tap water. The larval diet consisted of a homogenized suspension of liver powder, yeast, and rabbit chow. Overcrowding of larvae was prevented by rearing 250–300 larvae per pan.

Virus

The Zagazig hospital 501 (ZH-501) strain of RVF virus used in these studies was isolated from the serum of a fatal human hemorrhagic fever case in the Sharqiya governorate, Egypt, in 1977, and had undergone two passages in fetal rhesus monkey lung (FRhL) cells.[6] Approximately 500 aliquots of the stock virus (FRhL-2) were initially prepared, frozen, and stored at −70°C, thereby standardizing the source of virus throughout these experiments. The titer of the stock virus was $10^{7.7}$ plaque-forming units (PFU) per ml as measured in Vero C 1008 cell monolayers (ATCC # CRL 1586).

Infection of hamsters and mosquitoes

Rift Valley fever viremia profiles were determined in four groups of 90- to 100-g female Golden Syrian hamsters (Lakeview Colony, New Field,

N. J.) following intraperitoneal (IP) inoculation of 100–4,000,000 PFU of virus. Hamsters were bled at 4-hour intervals from the retroorbital plexus using heparinized capillary tubes. Blood samples (0.25 ml) were diluted in 1.0 ml of Medium 199 Hanks' balanced salt solution buffered to pH 7.4 with 1.10 g/liter of $NaHCO_3$ + 10% heat-inactivated (56°C for 30 minutes) fetal bovine serum (M199/10% FBS) and stored at −70°C until tested for virus.

To infect mosquitoes, hamsters were inoculated IP with approximately 10,000 PFU of RVF virus. Depending upon the desired titer, a viremic hamster was sedated at different intervals postinoculation by intramuscular injection of ketamine hydrochloride (100 mg/kg) (Vetalar® Parke-Davis) and xylazine (5 mg/kg) (Rompun® Haver-Lockhart) into the caudal thigh muscle. The hamster's abdomen and back were shaved before placing it in a 30.5 × 30.5 × 30.5 cm wire cage containing mosquitoes 5–8 days of age. The cage was kept in a dark humid atmosphere in a bioclimatic chamber and mosquitoes were exposed to the hamster for a 2-hour period. Pre- and post-mosquito exposure hamster blood samples were taken as described. To determine an infective dose (ID), or the amount of virus ingested by mosquitoes, freshly fed mosquitoes (usually 5) were collected, placed in individual micro-centrifuge tubes, and frozen at −70°C until tested for virus. The remaining mosquitoes were anesthetized with carbon dioxide and replete females were removed, counted, and then transferred to a new cage. Partially fed and unfed mosquitoes were removed and destroyed. Replete females were maintained in bioclimatic chambers at described conditions. To monitor virus replication, mosquitoes were collected and frozen at −70°C following different periods of extrinsic incubation.

Demonstration of transmission and infection

Following oviposition, randomly selected female mosquitoes were given the opportunity to refeed individually on 90- to 100-g female hamsters after 7, 10, and 12 days of extrinsic incubation. Mosquitoes were placed individually in 80 mm × 30 mm glass tubes. One end of the tube was covered with screening, and the other end was fitted with a plastic cap which had the center removed and replaced with two overlapping pieces of rubber. This self-closing cap permitted easy and safe insertion and removal of a mosquito. Hamsters

were placed individually in 130 mm × 40 mm perforated metal cylinders which were closed on one end. A glass tube containing a mosquito was then placed in the open end of the restrainer and pushed against the hamster. The tube was taped to the restrainer and an identification number was given to both the mosquito and the hamster.

Transmission experiments were conducted in a modified hospital-type infant incubator, located in a darkened room. The humidity was maintained between 70 and 90%, and the temperature varied between 25 and 29°C. After approximately 4 hours, the mosquitoes and hamsters were separated, and the hamsters were caged singly and observed daily for 27 days for clinical signs of illness and mortality. Virus transmission to a hamster was verified by the recovery of RVF virus from the liver of the dead animal. Serum was obtained from a random sample of surviving hamsters and assayed for RVF virus antibody. Each mosquito exposed to a hamster was visually examined without magnification for evidence of blood feeding and then placed individually in a microcentrifuge tube and frozen at −70°C until tested for virus.

Five individually caged RVF virus seronegative hamsters which had not been exposed to the virus or mosquitoes were maintained in the same room as the experimental hamsters during each experiment to monitor possible aerosol transmission of RVF virus.

For this study, the following two definitions are offered for clarification. The *infection rate* is the proportion of mosquitoes that contained virus, as determined at a specific time (in this case day 7 or later), following exposure to a viremic blood meal. The *transmission rate* is the proportion of infected mosquitoes that transmitted virus following refeeding on a susceptible host.

Viral assays

Mosquitoes were processed for virus isolation by triturating individual mosquitoes in 1.0 ml of M199/10% FBS + 100 units/ml penicillin, 100 μg/ml streptomycin, and 5 μg/ml fungizone (PSF). After centrifugation at 660 × g for 15 min at 4°C, the mosquito suspensions were frozen at −70°C until tested for virus.

Livers were removed aseptically from dead hamsters, ground into a 20% (w/v) suspension in M199/10% FBS/PSF, centrifuged as above, and then the supernatant was dispensed and frozen at −70°C until tested.

Samples (mosquito, hamster liver and blood) were tested for virus by the plaque assay technique in Vero cells. Serial 10-fold dilutions were made in M199/10% FBS/PSF, and samples (0.1 ml) were inoculated onto Vero cell monolayers in 12-well plates, adsorbed for 1 hour at 36°C, and overlayed with 0.75% agarose in Eagle's basal medium in Earle's salts with HEPES buffer (4 g/liter) + 7% FBS + PSF. Cell cultures were incubated in a humidified atmosphere of 5% CO_2 in air for 4 days for development of characteristic plaques. The cells were stained with another overlay of the above medium containing neutral red (330 μg/ml), incubated, and plaques were enumerated on the following day.

Virus identity was confirmed using RVF virus antiserum in the plaque reduction neutralization (PRN) test as described by Early et al.[7] and the agar gel diffusion test as described by Ibrahim and Sweet[8] and Tomori.[9] Sera obtained from hamsters surviving the transmission experiments were tested for RVF virus antibody by the PRN test as described by Peters and Slone,[10] and by the indirect fluorescent antibody technique as described by Tesh et al.[11]

Statistical procedures

Data were analyzed on the IBM 360/65 and DEC PDP 11/34 computers using various statistical procedures from a computerized biostatistical analysis library and SAS[12] programs.

The RVF viremia profiles of four groups of hamsters inoculated with graded doses of virus were analyzed by 1-way analysis of variance (ANOVA). If the F statistic from the ANOVA was significant, then the least significant difference (LSD) method[13] was used to compare each dosage group mean time to death (MTD) with every other group MTD.

The filial generations of mosquitoes were grouped according to the number of viral PFU ingested by the freshly fed mosquitoes. A high, medium, and low ID group was established based upon the behavior of the viremic hamsters and an ANOVA test of the ID titers. Hamsters in the high ID group exhibited decreased activity with decreased but not absent responsiveness to external stimuli. However, the animals were not yet in a moribund state. Hamsters in the middle and low ID groups exhibited normal activity. Data were then analyzed within and among each of the three groups.

Analysis of infection and transmission rates. The

association between the length of the extrinsic incubation and the RVF virus infection rate in each generation of mosquitoes was examined by the chi-square test of independence. Because the observed frequencies of the transmission rates were too small for the sampling distribution of the chi-square statistic, the association between the length of the extrinsic incubation and the transmission rate was examined by Fisher's exact test.[14] Infection and transmission abilities of sequential filial generations of *Cx. pipiens* were analyzed by trend analysis using the chi-square test of independent proportions.[13]

The infection and transmission percentages were then transformed by means of the arcsine or angular transformation.[15] Correlation and regression analyses were then performed on the arcsine transformed percentages. Furthermore, for these analyses the data were weighted according to the sample sizes of the various generations.

The associations between the initial ID groups and the later infection and transmission rates were examined by chi-square and Fisher's exact tests.

Analysis of viral titers. Uninfected mosquitoes were excluded from this portion of the analysis. A random multivariate analysis was used to examine the associations between the viral titers of infected mosquitoes that refed in the transmission experiments with the following five variables: the transmission status (transmitter vs. nontransmitter), the ID groups (high, medium, or low), the amount of feeding (partially fed vs. replete), the extrinsic incubation period (day 7, 10, or 12), and the number of filial generations since field collection (F_1 through F_{16}).

Nonsignificant elements were combined, and then the significant associations were further analyzed by other statistical procedures such as Kolmogorov-Smirnov (KS) tests for goodness of fit,[16] ANOVA, LSD, and *t*-tests.

Data were collected on mosquitoes that were able to transmit virus to hamsters by probing without obtaining a blood meal. The frequency data were analyzed by trend analysis using the chi-square test.[13] The viral titer data were analyzed by ANOVA, LSD, and *t*-tests.

RESULTS

Infection of hamsters

Hamsters had predictable viremia profiles with little variation between individuals at 4-hour intervals following inoculation of graded doses of
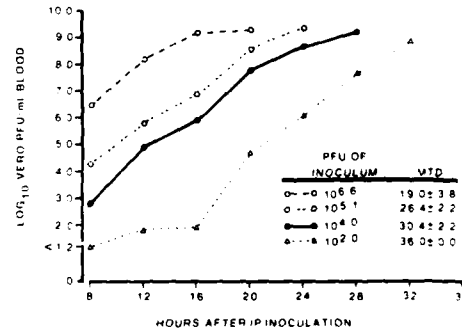

FIGURE 1. Rift Valley fever viremia profiles in hamsters following intraperitoneal inoculation of graded doses of ZH-501 strain of virus. MTD = mean time to death in hours ± standard deviation. Initial sample size for the group receiving $10^{6.6}$ PFU of virus was 4, and 5 for all other groups. All MTDs were significantly different from each other; the groups receiving $10^{5.1}$ and $10^{4.0}$ PFU were significant at $P < 0.05$, all other groups were significant at $P < 0.001$.

RVF virus. Most hamsters developed a viremia $\geq 10^{9.0}$ PFU/ml before dying, with survival time being dose dependent (Fig. 1). The shortest survival time was between 12 and 16 hours postinoculation with the highest dose ($10^{6.6}$ PFU) of virus. The lowest dose (100 PFU) of virus resulted in the longest survival time of between 32 and 36 hours. The mean times to death of hamsters from each of the four dosage groups were found to be significantly different from each other (Fig. 1).

Infection of mosquitoes

The number of viral PFU ingested by mosquitoes was regulated by exposing the mosquitoes to a viremic hamster at selected times postinoculation of virus. The specific times were determined by extrapolation from the viremia profiles in Figure 1. The titer of a viremic hamster and the titer in mosquitoes that fed on this hamster during a 2-hour period were significantly correlated ($r = 0.92$, $P < 0.001$). The titer of the freshly fed mosquitoes was usually 100-fold lower than the titer of the viremic hamster.

Confirmation of virus transmission to hamsters

Hamsters normally died from RVF virus 2-3 days following a successful mosquito transmission. No RVF virus antibody was detected in the sera from 300 randomly selected survivors. None

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TABLE 1

Rift Valley fever virus infection rates in 16 generations of Egyptian Culex pipiens, *and transmission rates to hamsters*

Infective dose group[*]	Generation[†]	Titer of infective dose[‡]	Infection rate		Transmission rate[§]	
			%	Proportion	%	Proportion[**]
High	1	7.2 ± 0.4	67	39/58	48	15/31
	2	7.3 ± 0.1	86	12/14	14	1/7
	3	7.1 ± 0.4	78	63/81	44	16/36
	5	6.2 ± 0.3	70	19/27	56	5/9
	8	7.2 ± 0.1	78	70/90	25	13/52[††]
	12	6.9 ± 0.3	93	169/181[††]	18	16/91
	14	6.6 ± 0.2	95	99/104	24	12/50
	16	7.1 ± 0.1	84	125/149	21	15/70
Medium	6	5.4 ± 0.1	83[‡‡]	19/23	17	2/12
	12	5.6 ± 0.2	87	84/97	12	6/49
	14	5.3 ± 0.1	92	136/148	27	18/66
Low	6	2.4 ± 0.1	51[‡‡]	26/51	5	1/19
	10	3.7 ± 0.4	57	54/94	17	5/30

[*] See text for behavior of viremic hamsters.
[†] Filial generation from field collection.
[‡] Geometric mean ± standard deviation, titer expressed as $\log_{10}$ PFU/sample. Sample sizes for high group generation 1, 3, 5, and 12 were 13, 29, 3, and 10, respectively, and 5 for all other generations.
[§] Transmission experiments done on days 7, 10, and 12 extrinsic incubation.
Number of mosquitoes that contained virus/number of mosquitoes tested.
[**] Number of mosquitoes that transmitted virus/number of infected mosquitoes that refed.
[††] Point at which there was a significant difference ($P < 0.005$) by chi-square test.
[‡‡] Significantly different from each other ($P < 0.01$) by chi-square test.

of the seronegative room control hamsters that were used for each experiment developed RVF virus antibody, and when challenged with the virus all hamsters died within 4 days.

Infection and transmission rates

Viral infection and transmission rates for 10 generations, representing F_1–F_{16}, of *Cx. pipiens* are shown in Table 1. The results were organized into a high, medium, or low group on the basis of the ID titer of the mosquitoes and the behavior of the viremic hamster. Replicate transmission experiments were done with generations 6, 12, and 14. There were no significant differences in the infection or transmission rates between days 7, 10, and 12 of extrinsic incubation; therefore, these data were combined.

For the mosquitoes in the high ID group, there were no significant differences between the infection rates from the F_1 through the F_8 generation. However, a significant change in the infection rate occurred between the F_8 and the F_{12}, when it increased from 78% to 93%, respectively (Table 1). The infection rate did not significantly change again between the F_{12} and the F_{16} generation. Filial generations 4, 7, 9, 11, 13 and 15 were not examined.

Within the same high ID group, there were no

significant differences between the transmission rates from the F_1 through the F_5 generation. A significant change did occur, however, in the transmission rate between the F_5 and the F_8 generation, when it decreased from 56% to 25%, respectively (Table 1). There were no other significant changes in the transmission rates between the F_8 and the F_{16} generations.

Significant changes in the infection or transmission rates did not occur between generations F_{6-14} in the middle ID group nor between generations F_6 and F_{10} in the low ID group (Table 1).

For the mosquitoes in the high ID group, an increase in filial generations resulted in a significant increase in the percent infected (Fig. 2A). However, the reverse was true with transmission, which significantly decreased as filial generations increased (Fig. 2B). The filial generations and percentage of mosquitoes infected in the medium ID group were also significantly correlated ($r = 0.89$, $P < 0.001$) and had a regression line similar to the high ID group. Transmission, however, was not correlated with the filial generations of the mosquitoes in the medium ID group.

Regardless of the initial ID, infection rates never reached 100%. This suggests that a proportion of individuals in every generation was refractory to infection (Table 1). Neither the infection nor the transmission rates significantly changed between
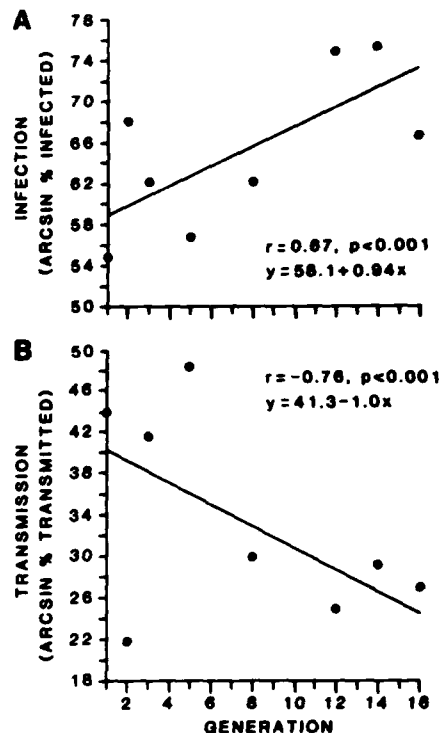
FIGURE 2. Infection (A) and transmission (B) rates as functions of filial generations of Egyptian *Cx. pipiens* which initially ingested a high infective dose of virus.

the high and medium ID groups for the F_{12} and F_{14} generations. However, the infection rate for the medium ID group was significantly higher than that for the low ID group in the F_6 generation (Table 1). Transmission rates for the F_6 generation in both the medium and low ID groups were not significantly different.

Viral titers

The initial random multivariate analysis showed significant differences between viral titers of infected mosquitoes that refed in the transmission experiments and the following three variables: the transmission status (transmitter vs. nontransmitter), the ID groups (high, medium, or low), and the number of generations since field collection. The mean viral titer of F_{14} generation mosquitoes was significantly higher ($P < 0.01$) than mean

viral titers of mosquitoes in the other generations. Since this difference (approximately 10-fold) could have been due to a laboratory error in the making or reading of viral dilutions, it was decided to eliminate all of the viral titer data from the F_{14} generation.

All the other data were then reanalyzed by a random multivariate analysis. The results of the second analysis showed no significant differences between the viral titers of mosquitoes and the number of generations from field collection (F_1 through F_{16}), the length of the extrinsic incubation period (day 7, 10, or 12), or the amount of feeding by the mosquitoes (37 partially fed vs. 368 replete). There were significant differences between the viral titers of transmitting and nontransmitting mosquitoes ($P < 0.0001$) and also between the viral titers of mosquitoes from the three ID groups ($P < 0.01$).

Rift Valley fever viral titers of 16 generations of infected *Cx. pipiens* that were involved in transmission experiments are shown in Table 2. Mean viral titers of transmitting mosquitoes from the three ID groups were not significantly different from each other. There was, however, a significant difference between the mean viral titers of nontransmitting mosquitoes from the high and medium ID groups. No significant differences were found between the mean viral titers of nontransmitting mosquitoes from the low ID group and those from the high and medium ID groups. Because of the significant difference between the medium and high ID groups the viral titer data could not be combined.

Viral titers of infected mosquitoes from the high ID group that refed in transmission experiments are shown in Figure 3. The distribution of viral titers for engorged infected mosquitoes from the middle and low ID groups showed a pattern similar to those in the high ID group. Two-sample KS tests demonstrated that transmitting and nontransmitting mosquitoes were separate populations. One-sample KS tests showed that each of these populations had a gaussian distribution. Further analysis by *t*-tests showed a significant difference between the mean viral titers of transmitting and nontransmitting mosquitoes in each of the three ID groups (Table 2).

Transmission of RVF virus was also accomplished by probing mosquitoes which were unsuccessful in obtaining a blood meal. The mean viral titers in PFU ± standard deviation and sample size for these mosquitoes in the three ID groups

TABLE 2

Rift Valley fever viral titers in 16 generations of Egyptian Culex pipiens that refed and transmitted virus compared to titers in mosquitoes that refed but failed to transmit virus

Group*	Gen †	Viral titers of transmitters‡			Viral titers of nontransmitters		
		No	Range	Mean	No.	Range	Mean
High	1	15	4.2–5.8	5.3	16	2.0–4.2	2.9
	2	1	—	5.1	6	1.8–4.3	3.0
	3	16	4.0–7.1	5.3	20	1.0–3.9	2.7
	5	5	4.8–5.8	5.4	4	2.4–3.7	3.1
	8	13	4.7–5 8	5.2	39	1.5–4.3	3.2
	12	16	5.0–6.0	5.3	75	0.8–5.4	3.1
	16	15	5.1–6.2	5.4	55	1.5–4.6	2.8
	Total	81	4.0–7.1	5.3 ± 0.5§	215	0.8–5.4	3.0 ± 0.8**
Medium	6	2	5.6–6.2	5.9	10	3.1–4.2	3.4
	12	6	5.3–6.1	5.5	43	0.8–4.7	3.6
	Total	8	5.3–6.2	5.6 ± 0.3	53	0.8–4.7	3.5 ± 0.7**
Low	6	1	—	5.1	18	2.1–5.3	3.6
	10	5	5.2–6.3	5.6	25	1.6–4.5	3.1
	Total	6	5.1–6.3	5.5 ± 0.5	43	1.6–5.3	3.3 ± 0.8

* Titer of infective dose and behavior of viremic hamsters as in Table 1
† Filial generation from field collection
‡ Transmission experiments done on days 7, 10, and 12 extrinsic incubation, viral titers expressed as $\log_{10}$ PFU/sample.
§ Geometric mean ± standard deviation
Significant difference ($P < 0.0001$) by t-test in viral titers between transmitting mosquitoes and counterpart group that did not transmit
** Significantly different from each other by ANOVA ($P < 0.0001$ and LSD ($P < 0.01$)

were: high, $10^{5.4} \pm 0.4$ (n = 16); medium, $10^{5.5}$ (n = 1); low, $10^{5.4} \pm 0.1$ (n = 2). The number of these mosquitoes was not significantly associated with the number of generations since field collec-
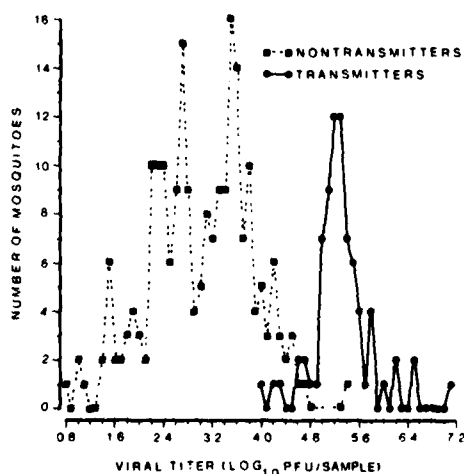

FIGURE 3. Distribution of Rift Valley fever viral titers in engorged transmitting and nontransmitting Egyptian *Cx. pipiens* which initially ingested a high infective dose of virus.

tion. No significant associations were found between the mean viral titers of these probing transmitting mosquitoes and the various generations or between the three ID groups. Comparisons of mean viral titers between engorged (Table 2) and probing transmitting mosquitoes within each of the ID groups were not significant.

Rift Valley fever virus replication in *Cx. pipiens* is shown in Figure 4. An eclipse phase consisting of a 32- to 316-fold decrease in viral titer was observed on day 2 postinfectious blood meal. A single line was not drawn through the mean of all data points for each day of extrinsic incubation because previous results indicated that there were three significantly distinct subpopulations of *Cx. pipiens*: uninfected individuals (Table 1) and two types of infected mosquitoes—transmitters and nontransmitters (Fig. 3 and Table 2). Therefore, individual mosquitoes were assigned to one of the three subgroups on the basis of their viral titer and a mean was calculated for that subgroup.

DISCUSSION

Laboratory colonization of field collected Egyptian *Cx. pipiens* mosquitoes resulted in significant changes in their infection and transmission rates of RVF virus (Table 1). The viral titers of trans-
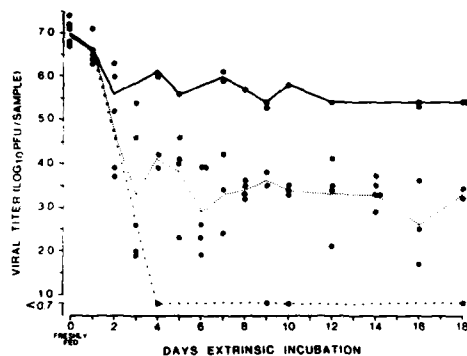
FIGURE 4. Rift Valley fever viral replication in *Cx. pipiens* collected from Sharqiya, Egypt. Uninfected ------ Hypothetical subpopulations: transmitters ———, nontransmitters - - - - -.

mitting and nontransmitting mosquitoes, however, did not significantly change from the F_1 through the F_{16} generation (Table 2). Meegan et al.[4] mentioned that laboratory colonization caused a decrease in the ability of this mosquito to transmit RVF virus, but data to support this observation were not presented. Their report on the infection and transmission rates of RVF virus by the F_4 generation of *Cx. pipiens* was similar to the results found in this study for comparable generations. Apparently laboratory colonization was inadvertently selecting mosquitoes which were able to become infected with virus (Fig. 2A) while at the same time were refractory to the spread of virus to the salivary glands (i.e., decreasing transmission rate, Fig. 2B).

Laboratory colonization has been reported to have a diverse and unpredictable effect on the infection and transmission of La Crosse virus by *Aedes triseriatus*.[17] Colonization has also been reported to alter the photoperiodic response for induction of egg diapause in this species.[18] There was no difference, however, reported for the infection and transmission of two enzootic strains of Venezuelan encephalitis (VE) virus by natural populations and subsequent F_2 and F_4 generations of *Culex taeniopus*.[19] It would be interesting to determine whether continued colonization of *Cx. taeniopus* would alter the infection and transmission rates of VE virus.

It is not surprising that laboratory and field populations of mosquitoes respond differently to various stimuli. It has been reported that when mosquitoes are removed from the field and placed in the laboratory they undergo drastic selection, with rapid alteration of the gene pool.[20] Stress factors such as overcrowding and underfeeding have been reported to be among the major reasons why insect populations kept in the laboratory for any length of time may undergo evolutionary change.[21] Also, the founder effect may account for differences observed between natural and laboratory populations of a species.[22]

It has been reported that the percentage of mosquitoes infected, and the efficiency of subsequent transmission of virus, varied directly with the amount of virus ingested by the mosquitoes.[23-27] We found a similar result in the infection of an early generation of *Cx. pipiens* with RVF virus. The F_6 generation mosquitoes that initially received a low ID of virus had a significantly lower infection rate than their cohorts that received a medium ID of virus (Table 1). The transmission rates, however, were not significantly different for the F_6 generation tested at these two ID levels. This lack of significance between the transmission rates could be related to the small sample sizes (Table 1).

No significant differences were found in either the infection or transmission rates in late generations (F_{12} and F_{14}) of mosquitoes that were tested at both the high and medium ID groups (Table 1). Unfortunately, we were unable to test the early generations of mosquitoes at these two ID levels. There is the possibility that the similarity within the infection and transmission rates observed for these two ID groups may not have field applicability because these rates were derived from late generation mosquitoes which had significantly changed from the F_1 generation mosquitoes. However, it is also possible that selective physiological responses of mosquitoes to viral infections do not occur when they have ingested over 10^5 PFU of virus.

Viral titers of transmitting females were similar to each other regardless of the amount of virus the mosquitoes initially ingested. We were surprised to find that the initial ID produced significant differences in the mean viral titers of nontransmitting females. The nontransmitters from the high ID group had a significantly lower mean viral titer than the nontransmitting mosquitoes from the medium ID group (Table 2). This finding is perhaps similar to the report by Kramer et al.,[25] which demonstrated that viral titers decreased in the mesenterons of mosquitoes that were infected with high viral doses.

Following ingestion of virus, *Cx. pipiens* could be separated into three significantly distinct subpopulations. One subpopulation was refractory to infection with virus. This group apparently had a very strong mesenteronal infection barrier because infection rates never reached 100% regardless of the initial ID (Table 1). There were two subpopulations of mosquitoes which became infected with virus; transmitters and nontransmitters. Viral titers of the transmitting females were usually 100-fold higher than the viral titers of nontransmitting females (Table 2). Significant differences between the viral titers of transmitting and nontransmitting mosquitoes have been reported by others, and demonstrate that not every infected female mosquito is a competent vector.[25, 28, 29]

There was some overlap between the viral titers of transmitting and nontransmitting mosquitoes (Table 2, Fig. 3). The few high titered, engorged, nontransmitting mosquitoes may represent a salivary gland infection barrier as described by Kramer et al.[25]

Because the distributions of the viral titers of transmitting and nontransmitting mosquitoes were significantly different from each other (Fig. 3), it was possible to predict the vector potential of most of the mosquitoes by their viral titer (Fig. 4).

Transmission of RVF virus was also accomplished by females which had probed for a blood meal but were not visibly engorged. This type of virus transmission has been observed with other viruses.[23, 29, 30] It is possible that if this behavior occurs in nature, the infected unfed probing females could transmit RVF virus to a larger number of susceptible animals or humans than those females which were successful in blood feeding on their first attempt.

In nature, humans and domestic animals frequently circulate RVF viremias ranging from $10^{8.6}$–$10^{10.0}$ mouse intracerebral lethal dose 50/ml serum, respectively.[31-33] Therefore, using hamsters circulating a high titer of virus as animal models for infecting potential vectors is relevant and realistic in studying RVF. The predictable death pattern in RVF virus-infected hamsters also makes this animal an excellent recipient host for transmission studies.

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